The objection to the specification as allegedly making contradictory statements at pages 5 and 6 of the specification with respect to the correlation of BAG expression levels to increased or decreased risk of cancer spread, is respectfully traversed. The Examiner has requested clarification. It is respectfully submitted that Applicant's specification, at page 5, lines 14-18, describes the embodiment of Applicant's invention where high BAG-1 expression levels correlates to decreased risk of tumor recurrence or spread. This embodiment is exemplified with respect to the correlation of high BAG-1 expression levels to decreased risk of tumor recurrence or spread for breast cancer, as set forth in the Examples of the specification at pages 32-26.

It is respectfully submitted that Applicant's specification, at page 6, lines 5-7, describes the embodiment of Applicant's invention where high BAG-1 expression levels correlates to increased risk of tumor recurrence or spread. This embodiment is exemplified with respect to the correlation of high BAG-1 expression levels to increased risk of tumor recurrence or spread for prostate cancer, as set forth in the Rule 132 of Dr. Reed submitted herewith as Attachment A. As set forth in Dr. Reed's declaration, at page 4, "tumor-specific increases in cytosolic BAG1 and nuclear BAG1L levels commonly occur in prostate cancers; ...immunohistochemical analysis of BAG1N (cytosolic) and BAG1L (nuclear) expression provides prognostic information about prostate cancer patients, including information about progression to hormone-refractory disease; and...unlike the situation with breast cancer, higher levels of cytosolic BAG1N

expression are associated with unfavorable prognosis rather than favorable prognosis."

With respect to what isoform of BAG-1 is bound by the BAG-1 antibody, it is respectfully submitted that, as set forth in Takayama et al. (1998), Cancer Research, **58**:3116-3131, at the paragraph bridging pages 3123 and 3125, the anti-BAG-1 antibody used recognizes all three isoforms of BAG-1. Accordingly, reconsideration and withdrawal of this objection to the specification is respectfully submitted.

The rejection of claims 9-10, 18 and 47 under 35 U.S.C. §112, second paragraph, as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention, is respectfully traversed.

With respect to the Examiner's concern related claims 9 and 10, Applicant's specification, at the sentence bridging pages 10 and 11, teaches:

> The polypeptide sequences for human BAG-1N, BAG-1M, and BAG-1L are 230, 274 and 345 amino acids in length, respectively.

Takayama et al. (1998), Cancer Research, **58**:3116-3131 (cited in the specification at p. 10, lines 19-20), at Figure 3C discloses that human BAG-1L is 345 amino acids, BAG-1M is 274 amino acids and that BAG-1 is 230 amino acids. Thus, it is respectfully submitted that one of skill in the art would readily understand, in view of Applicant's specification, that BAG-1N corresponds to

the 230 amino acid isoform referred to as BAG-1 in Figure 3C of Takayama et al.

It is respectfully submitted that the Examiner's concern regarding the limitation "said BAG protein" in claim 18 has been rendered moot by the amendment to claims 16 and 18 herewith, such that the term protein has been deleted.

Applicant respectfully disagrees with the Examiner's assertion at p. 3, lns. 8-9, of Official Action Paper No. 4, that:

> Claim 47 is unclear in that it fails to correlate how the comparison of BAG expression levels indicates effectiveness of treatment.

Applicant's specification, at p. 29, ln. 6 through p. 30, ln. 5, teaches those of skill in the art that:

> As used in the context of a course of treatment, **"effectiveness" refers to the ability of the course of treatment to decrease the risk of tumor recurrence or spread and therefore to increase the likelihood of disease-free or overall survival of the patient.** This method will have particular utility when the level of BAG expression in the tumor cells of a patient is abnormal compared to the level of BAG expression in the non-tumor cells of the patient. **Comparison of the first and second BAG expression levels will thereby serve to indicate whether BAG expression level is returning to that of non-tumor cells, implying a more effective course of treatment, or whether BAG expression level is remaining abnormal or increasing in abnormality, implying a less effective course of treatment.** Levels of BAG expression may be determined using a plurality of samples from the patient, as described herein. A preferable sample form for this

> embodiment shall be a body fluid sample, such as serum sample, an exudate sample, and the like.
>
> This embodiment of the invention is particularly useful when combined with the method of determining the risk of tumor recurrence or spread in a cancer patient, and thereby determining the proper course of treatment in a cancer patient. **Specifically, the proper course of treatment of a cancer patient may be determined by determining the level of BAG expression in a sample from a patient, then classifying the patient's likelihood of disease-free or overall survival according to the level of BAG expression. The course of treatment may then be monitored, according to the present embodiment of the invention, on one or more occasions to determine the effectiveness of the course of treatment.** (Emphasis added)

It is respectfully submitted that, in view of Applicant's specification, one of skill in the art would readily understand that detecting changing levels of BAG gene expression in a particular patient's cancerous sample would be indicative of the effectiveness of that particular treatment. Accordingly, reconsideration and withdrawal of this rejection is respectfully requested.

The rejection of claims 16-37, and 44-45 under 35 U.S.C. §112, first paragraph, as allegedly lacking enablement, is respectfully traversed. Although Applicant does not concede the merits of this rejection, in order to expedite prosecution, it is respectfully submitted that this rejection has been rendered moot by the amendments to claims 16, 25, 27, 34, and 44 herein, which amendments require that the cancer is breast cancer.

Accordingly, reconsideration and withdrawal of this rejection is respectfully requested.

The rejection of claim 23 under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains to make and/or use the invention, is respectfully traversed. Applicant respectfully disagrees with the Examiner's assertion that "the specification specifically sets forth that in vitro reference levels are unreliable (see page 18, 2nd paragraph)."

Contrary to the Examiner's assertion, Applicant does not state that all in vitro reference levels obtained from cultured cells are unrealiable. Rather, Applicant merely teaches that it is not necessary that all reference levels need to be derived from in vitro cultured cells. For example, Applicant's specification, at p. 18, lns. 8-13, clearly provides guidance and support to those of skill in the art for the use of in vitro-derived cell culture reference levels in the claimed methods by clearly teaching that:

> The reference level may also be a level of BAG expression of *in vitro* cultured cells which may or may not have been manipulated to simulate tumor cells, or may have been manipulated in any other manner which yields expression levels which accurately determine the reference level.

Accordingly, reconsideration and withdrawal of this rejection is respectfully requested.

The rejection of claim 47 under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains to make and/or use the invention, is respectfully traversed. Applicant respectfully disagrees with the Examiner's assertion that:

> Claim 47 recites a method of determining effectiveness of treatment by measuring BAG gene expression levels before and after the treatment. **The specification fails to provide any guidance**...that such a comparison would enable a determination of treatment effectiveness. (Emphasis added)

As set forth above, Applicant's specification, at p. 29, ln. 6 through p. 30, ln. 5, provides clear guidance to those of skill in the art as follows:

> As used in the context of a course of treatment, **"effectiveness" refers to the ability of the course of treatment to decrease the risk of tumor recurrence or spread and therefore to increase the likelihood of disease-free or overall survival of the patient.** This method will have particular utility when the level of BAG expression in the tumor cells of a patient is abnormal compared to the level of BAG expression in the non-tumor cells of the patient. **Comparison of the first and second BAG expression levels will thereby serve to indicate whether BAG expression level is returning to that of non-tumor cells, implying a more effective course of treatment, or whether BAG expression level is remaining abnormal or increasing in abnormality, implying a less effective course of treatment.** Levels of BAG expression may be determined using a plurality of samples from the patient, as described herein. A preferable sample form for this embodiment shall be a body fluid sample, such as serum sample, an exudate sample, and the like.

> This embodiment of the invention is particularly useful when combined with the method of determining the risk of tumor recurrence or spread in a cancer patient, and thereby determining the proper course of treatment in a cancer patient. **Specifically, the proper course of treatment of a cancer patient may be determined by determining the level of BAG expression in a sample from a patient, then classifying the patient's likelihood of disease-free or overall survival according to the level of BAG expression. The course of treatment may then be monitored, according to the present embodiment of the invention, on one or more occasions to determine the effectiveness of the course of treatment.** (Emphasis added)

It is respectfully submitted that one of skill in the art, in view of the above-quoted guidance provided in Applicant's specification, would have a reasonable expectation of success of determining the effectiveness of a particular cancer treatment by comparing BAG expression levels before and during treatment. Accordingly, reconsideration and withdrawal of this rejection is respectfully requested.

The rejection of claims 48 and 49 under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains to make and/or use the invention, is respectfully traversed. Applicant respectfully disagrees with the Examiner's assertion that:

> Claims 48 and 49 recite determining prognosis of disease free or overall survival by determining BAG activity. **The specification fails to provide any guidance**...that BAG activity would enable a

> determination of prognosis of disease free or overall survival. (Emphasis added)

As set forth in the specification, at the paragraph bridging pages 30 and 31, Applicant provides clear guidance to those of skill in the art as follows:

> As used herein, "BAG activity level" or level of "BAG activity" refers to the level of active, uninhibited BAG polypeptides present in tumor cells or body fluid, and the degree of activity of these polypeptides. Hence, level of activity shall be influenced by a plurality of factors including: levels of BAG, presence of less active or more active forms (including isoforms) of BAG, presence of less active or more active mutants of BAG, presence of proteins or other molecules which increase BAG activity, presence of proteins or other molecules which decrease BAG activity. For example, levels of an antagonist to BAG, such as Hip (an antagonist to BAG-1, see for example Hohfeld and Jentsch 1997, EMBO J 16:6029-6216), will be understood to commensurately lower BAG activity even if BAG protein levels remain unchanged. Determination of BAG activity shall therefore be carried out by a plurality of methods, including: assay of biological activity of BAG (such as ability to prevent cell death or other apoptotic assays, see, for example, United States Patent 5,550,019), assay of BAG protein levels, assay of the mRNA encoding a BAG protein, assay of the DNA which constitutes a BAG gene, assay of proteins (or the mRNA or DNA encoding the proteins) or other molecules which increase or decrease BAG activity, and any combination thereof. (Emphasis added)

It is respectfully submitted that one of skill in the art, in view of the above-quoted guidance provided in Applicant's specification, would have a reasonable expectation of success of determining the prognosis of disease free or overall survival of a patient suffering from cancer by determining the BAG activity

levels of those patients. Accordingly, reconsideration and withdrawal of this rejection is respectfully requested.

The rejection of claims 1-2, 5-11, 13, 15-17, 19-21, 24-28, 32-32, 34-36 and 42-46 under 35 U.S.C. §102(a) as allegedly anticipated by Krajewski et al., (March/1999) Endocrine Related Cancer, **6(1)**:29-40, is respectfully traversed. It is respectfully submitted that this rejection has been rendered moot by the Katz-type Rule 132 Declaration of Dr. Reed submitted herewith as Attachment B. Specifically, at p. 2, paragraph 4 of the Rule 132 Declaration, Dr. Reed declares that "the coauthors of the Krajewski et al. reference did not contribute to aspects of the subject matter which are described and claimed in the above-referenced application." Therefore, Krajewski et al. is not applicable as prior art. Accordingly, reconsideration and withdrawal of this rejection is respectfully requested.

The rejection of claims 1, 3-4, 6-11, 13-15, 42-43 and 45-46 under 35 U.S.C. §102(a) as allegedly anticipated by Tang et al., (June/1999) J. Clin. Oncology, **17(6)**:1710-1719, is respectfully traversed. It is respectfully submitted that the effective publication date of this journal publication is June 17, 1999, which is the date Tang et al. was received via mail by UCSD's Biomedical Library (see Attachment D showing the date of receipt by UCSD). It is respectfully submitted that this rejection has been rendered moot by the Rule 131 Declaration of Dr. Reed submitted herewith as Attachment C. Specifically, at p. 2, paragraph 5 of the Rule 131 Declaration, Dr. Reed declares that "prior to June 17 1999, I established the correlation that

'patients whose tumors contain elevated levels of cytosolic BAG-1 protein are more likely to enjoy long-term survival and freedom from distant metastases, compared to those with BAG-1 negative turmors.'" Therefore, Tang et al. is not applicable as prior art. Accordingly, reconsideration and withdrawal of this rejection is respectfully requested.

The rejection of claims 1, 3-4, 6-11, 13-15, 42-43 and 45-46 under 35 U.S.C. §102(b) as allegedly anticipated by Zapata et al., (1998) Breast Cancer Research and Treatment, **47**:140, is respectfully traversed. Applicant's invention distinguishes over the Zapata reference by requiring a method for determining a prognosis of disease free or overall survival in a patient suffering from cancer, said method comprising:

(a) determining a BAG gene expression level in a cancerous tissue sample or body fluid from said patient; and

(b) classifying said patient as belonging to either a first or second group of patients, wherein said first group of patients having high levels of expression of the BAG gene is classified as having a different likelihood of suffering tumor recurrence or spread than said second group of patients having low levels of expression of the BAG gene.

After discussing at length and in detail numerous results related to Bcl-2, p53, Bax and Bcl-X_L, the Zapata reference merely states, at p. 138, col. 1, 3rd paragraph, that: "Interestingly, the intensity of BAG-1 immunostaining was often higher in invasive cancers compared to normal epithelium." However, Zapata goes on to acknowledge, at p. 138, col. 2, lns. 1-3, that:

> How these various activities of the BAG-1 protein **impact on the pathogenesis and progression of breast cancers remains to be determined.** (Emphasis added)

Such disclosure related to the uncertainties of the impact of BAG-1 on the progression of breast cancer does not teach each element of Applicant's claimed method for determining a prognosis of disease free or overall survival in a patient suffering from cancer. Accordingly, reconsideration and withdrawal of this rejection is respectfully requested.

The rejection of claims 1-2, 5-13, 15-21, 24-36 and 38-46 under 35 U.S.C. §103(a) as allegedly obvious over by Krajewski et al., supra, in view of United States patents issued to Sano et al., US Patent 5,665,539 (March/1999), An et al., US Patent 5,882,864 (March/1999) or Ravdin et al., US Patent 5,862,304 (Jan./1999), is respectfully traversed. It is respectfully submitted that this rejection has been rendered moot by the Katz-type Rule 132 Declaration of Dr. Reed submitted herewith as Attachment B, which has removed the primary Krajewki et al. reference from prior art. Accordingly, reconsideration and withdrawal of this rejection is respectfully requested.

The rejection of claims 1, 3-4, 6-15, 38-43 and 45-46 under 35 U.S.C. §103(a) as allegedly obvious over Tang et al., supra, in view of Sano et al., US Patent 5,665,539 (March/1999), is respectfully traversed. It is respectfully submitted that this rejection has been rendered moot by the Rule 131 Declaration of Dr. Reed submitted herewith as Attachment C, which has removed the primary Tang et al. reference from prior art. Accordingly,

reconsideration and withdrawal of this rejection is respectfully requested.

The rejection of claims 1, 3-4, 6-15, 38-43 and 45-46 under 35 U.S.C. §103(a) as allegedly obvious over by Zapata et al., supra, in view of Sano et al., US Patent 5,665,539 (March/1999), is respectfully traversed. As set forth above, Zapata merely describes, at p. 138, col. 2, lns. 1-3, that:

> How these various activities of the BAG-1 protein **impact on the pathogenesis and progression of breast cancers remains to be determined.** (Emphasis added)

Moreover, Zapata discloses, at p. 137, sentence bridging cols. 1 and 2, that:

> The multitude of apoptosis-regulating proteins expressed in these tumor cells and the complex interactions among these proteins suggest that the relative resistance to apoptosis in individual cases of breast cancer **will be difficult to predict from merely monitoring whether or not any particular one of these proteins is present.** (Emphasis added)

Thus, Zapata alone, or in combination with Sano et al., does not disclose or suggest using the single BAG-1 protein for determining a prognosis of disease free or overall survival in a patient suffering from cancer.

The secondary reference Sano et al. is unable to cure the deficiencies of the primary Zapata reference because it merely teaches immuno-PCR detection techniques. It is respectfully submitted that because the results described by Zapata were uncertain and preliminary, and because Sano et al. is

merely related to immuno-PCR detection technuiques, one of ordinary skill in the art would not have been motivated to combine Sano et al. with Zapata et al. Moreover, the combination of Zapata and Sano does not teach or suggest Applicant's claimed methods of determining BAG-1 protein expression levels for determining a prognosis of disease free or overall survival in a patient suffering from cancer. Accordingly, reconsideration and withdrawal of this rejection is respectfully requested.

CONCLUSION

In light of the Amendments and Remarks herein, Applicants submit that the claims are now in condition for allowance and respectfully request a notice to this effect. Should the Examiner have any questions, he/she is invited to call the undersigned attorney.

Respectfully submitted,

August 10, 2000
Date

Robert T. Ramos
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Telephone No. (858) 535-9001
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4370 La Jolla Village Drive
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San Diego, California 92122
USPTO CUSTOMER NO. 23601

ATTACHMENTS:

Attachment A- Rule 132 Declaration by Dr. Reed
Attachment B- Rule 132 Declaration by Dr. Reed
Attachment C- Rule 131 Declaration by Dr. Reed
Attachment D- Cover page of the J. of Clinical Oncology 17(6) showing receipt by UCSD on June 17, 1999.

ATTACHMENT A: DECLARATION UNDER 37 CFR 1.132
P-LJ 3578
Serial No.: 09/350,518

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, Washington, D.C. 20231, on August 10, 2000.

By _____
Robert T. Ramos, Reg. No. 34,949

August 10, 2000
Date of Signature

PATENT
Our Docket: P-LJ 3578

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: John C. Reed

Serial No: 09/350,518

Filed: July 9, 1999

For: A METHOD FOR DETERMINING THE PROGNOSIS OF CANCER PATIENTS BY MEASURING LEVELS OF BAG EXPRESSION

Group Art Unit: 1642

Examiner: J. Nichols

Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

CONSIDERED 11/17/00

I, John C. Reed, hereby declare as follows:

1. I am the John C. Reed who is named as a sole inventor on the above-identified patent application.

2. I understand that the Examiner has requested clarification of the embodiments of the claimed invention described at pages 5 and 6 of the specification.

3. The embodiment described at page 5 of the specification relates to the discovery that overproduction or a high level of expression of a BAG gene correlates to patients having a decreased risk of tumor recurrence or spread. An

ATTACHMENT A

example of such correlation to breast cancer is set forth in the Examples of the specification at pages 32-36.

4. The embodiment described at page 6 of the specification relates to the correlation of high levels of expression of a BAG gene to patients having an increased risk of tumor recurrence or spread. An example of such correlation to prostate cancer is set forth as follows.

5. Immunohistochemical methods were used to evaluate the expression of the BAG1N (cytosolic) and BAG1L (nuclear) proteins in primary and metastatic prostate cancer specimens. Previously it was determined that BAG1N is cytosolic while BAG1L is nuclear (BAG1N is referred to as BAG-1 in Takayama et al. 1998, Cancer Res. **58**:3116-3131) and (Packham et al. 1997, Biochem. J. **328**:807-813). Using anti-BAG1 monoclonal antibodies (Takayama et al. 1998, supra), immunohistochemical methods and archival paraffin-embedded prostate cancer specimens were used to evaluate the expression of the nuclear (BAG1L) and cytosolic (BAG1N) proteins in over 800 cases of prostate cancer. Comparisons were made with BAG1 immunostaining results in normal prostate and benign prostatic hypertrophy. Tissue microarray technology was exploited for much of this analysis, permitting the analysis large numbers of tumor specimens.

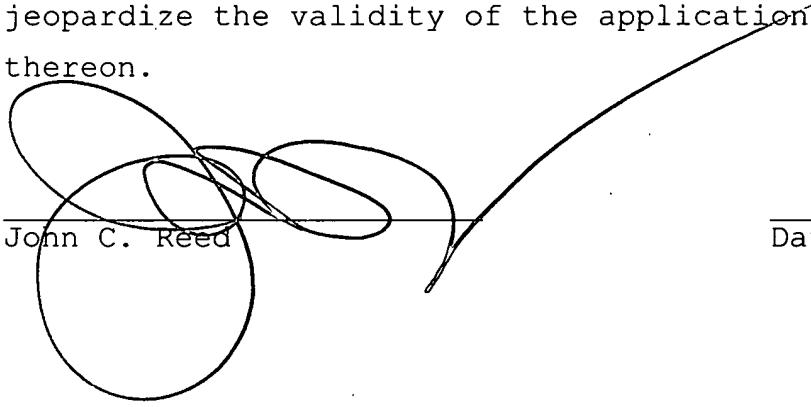
6. Compared to normal prostate, cytosolic BAG1N immunostaining was elevated in 746 of 876 (85%) of prostate cancers. Nuclear BAG1 (BAG1L) immunostaining was inappropriately increased in 171 of 676 (25%) of prostate cancers, compared to

normal prostate gland epithelium. Clinical follow-up data or other types of laboratory information were available for some of these patients, demonstrating a variety of correlations of BAG1 expression with more aggressive tumor phenotypes. For example, in a cohort of 62 patients with early-stage (T1,T2) disease and low Gleason grade (gr 2-6), higher percentages of BAG1 immunopositive tumor cells were associated with higher PSA levels prior to radiation therapy ($p = 0.05$), and with a higher incidence of distant metastases after therapy ($p = 0.05$). Higher intensity BAG1 immunostaining was also associated with a higher incidence of metastatic relapse after radiation therapy ($p < 0.0001$). In addition, immunohistochemical analysis of 722 prostate cancer specimens in a microarray format revealed higher percentages of BAG1 immunopositive cells in tumors ($n = 722$) compared to normal prostate ($n=54$) : mean + SE: 41 + 3% normal versus 78 + 1 % cancer ($p<0.0001$). An association was also identified between higher percentages of BAG1 immunopositive tumor cells and locally advanced disease ($p = 0.05$) ($n = 625$ patients) and with hormone refractory disease ($p<0.001$) ($n = 263$ patients).

7. Higher percentages of tumor cells with nuclear BAG1-immunostaining (BAG1L) as well as higher intensity nuclear BAG1L (BAG1L intensity) staining were also associated with hormone-refractory disease: $p<0.001$ and $p<0.0001$, respectively ($n=263$). Higher intensity BAG1 nuclear immunostaining was also correlated with hormone-refractory (HR) disease in a cohort of 92 prostate cancer patients with locally-advanced disease who were treated with anti-androgen therapy prior to surgery (50% vs 9% HR; $p<0.001$).

8. From these results, it can be concluded that tumor-specific increases in cytosolic BAG1N and nuclear BAG1L levels commonly occur in prostate cancers; that immunohistochemical analysis of BAG1N (cytosolic) and BAG1L (nuclear) expression provides prognostic information about prostate cancer patients, including information about progression to hormone-refractory disease; and that, unlike the situation with breast cancer, higher levels of cytosolic BAG1N expression are associated with unfavorable prognosis rather than favorable prognosis.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



John C. Reed

8-7-00
Date

ATTACHMENT B: DECLARATION UNDER 37 CFR 1.132
P-LJ 3578
Serial No.: 09/350,518

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, Washington, D.C. 20231, on August 10, 2000.

By _____
Robert T. Ramos, Reg. No. 34,949

August 10, 2000
Date of Signature

PATENT
Our Docket: P-LJ 3578

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
John C. Reed

Serial No: 09/350,518

Filed: July 9, 1999

For: A METHOD FOR DETERMINING THE PROGNOSIS OF CANCER PATIENTS BY MEASURING LEVELS OF BAG EXPRESSION

Group Art Unit: 1642

Examiner: J. Nichols

Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

I, John C. Reed, hereby declare as follows:

Considered 11/17/00

1. I am the John C. Reed who is named as a sole inventor on the above-identified patent application.

2. I am the John C. Reed who is named as a co-author with S. Krajewski, M. Krajewska, B.C. Turner, C. Pratt, B. Howard, J. M. Zapata, V. Frenkel, S. Robertson, Y. Ionov, H. Yamamato, M. Perucho and S. Takayama, on the reference entitled "Prognostic Significance Of Apoptosis Regulators In Breast Cancer" published as Krajewski et al., Endocrine-Related Cancer, 1999, 6:29-40.

ATTACHMENT B

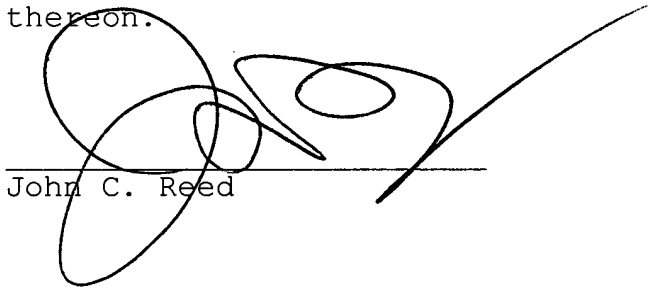
3. The invention claimed in the subject application was conceived solely by me and reduced to practice in my laboratory by me or persons acting under my direction and supervision.

4. Some of the subject matter of the Krajewsi et al., supra, reference described at Figure 3 and p. 36 is related to the subject matter described and claimed in the above-identified patent application. The one experiment described at Figure 3 and p. 36 in the Krajewski et al. 1999 related to the claimed invention was performed under my direction and supervision or persons acting under my direction and supervision. Therefore, the coauthors of the Krajewski et al. reference did not contribute to aspects of the subject matter which are described and claimed in the above-referenced application.

5. It is respectfully submitted that the named co-authors of the Krajewski et al. 1999 publication, S. Krajewski, M. Krajewska, B.C. Turner, C. Pratt, B. Howard, J. M. Zapata, V. Frenkel, S. Robertson, Y. Ionov, H. Yamamato, M. Perucho and S. Takayama, did not contribute to the initial conception or any of the subsequent conceptual aspects of the subject matter which is described and claimed in the above-referenced application. Therefore, S. Krajewski, M. Krajewska, B.C. Turner, C. Pratt, B. Howard, J. M. Zapata, V. Frenkel, S. Robertson, Y. Ionov, H. Yamamato, M. Perucho and S. Takayama were not named as co-inventors of the claimed invention.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on

information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



John C. Reed

7-7-00

Date

ATTACHMENT C: DECLARATION UNDER 37 CFR 1.131
P-LJ 3578
Serial No.: 09/350,518

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, Washington, D.C. 20231, on August 10, 2000.

By _____
Robert T. Ramos, Reg. No. 34,949

August 10, 2000
Date of Signature

PATENT
Our Docket: P-LJ 3578

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: John C. Reed	)	Group Art Unit: 1642
Serial No: 09/350,518	)	Examiner: J. Nichols
Filed: July 9, 1999	)	
For: A METHOD FOR DETERMINING THE PROGNOSIS OF CANCER PATIENTS BY MEASURING LEVELS OF BAG EXPRESSION	)	

Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. § 1.131

Sir:

1. I, John C. Reed, am the sole inventor named on the above-identified patent application.

2. I have reviewed the Office Action mailed February 10, 2000, in connection with the above-identified application. I understand that the Tang et al., (1999) reference, J. Clinical Oncology, **17(6)**:1710-1719 (referred to herein as "Tang et al.") has been cited against the pending claims as allegedly anticipating, or in the alternative rendering the pending claims obvious.

Considered 11/17/00

3. I respectfully submit that I conceived and reduced to practice in the United States of America the claimed invention prior to the June 17, 1999 general publication date of Tang et

al. The June 17, 1999 publication date was determined based on the date the journal publication was received by the University of California, San Diego BioMedical Library.

4. As evidence that I conceived and reduced to practice the claimed invention prior to the publication date of Tang et al., a photocopy of the relevant portions of a manuscript titled "BAG-1: A Novel Biomarker Predicting Long-term Survival in Early-Stage Breast Cancer" drafted and created prior to June 17, 1999, is provided herewith as Exhibit A.

5. Exhibit A shows the relevant experiments and analysis conducted prior to June 17, 1999, set forth in the Example section of the specification at pages 32-36. As a result of this analysis, as set forth in Exhibit A at p. 6, lns. 3-6, prior to June 17 1999, I established the correlation that "patients whose tumors contain elevated levels of cytosolic BAG-1 protein are more likely to enjoy long-term survival and freedom from distant metastases, compared to those with BAG-1 negative turmors" (see Exhibit A).

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the

United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Respectfully submitted,

8-7-00
Date

John C. Reed

One of every nine women currently develops breast cancer at some point in her life. Among women with early-stage breast cancers treated with lumpectomy and local radiotherapy, 10-20% will experience local recurrences and 30-40% will develop distant metastatic disease which is often fatal (1). The overexpression of the HER2/Neu oncoprotein has been shown to predict breast cancer patients at risk for metastatic disease and novel therapeutic strategies have been developed to target this receptor (2). A need exists for identifying other prognostic markers that accurately predict long-term outcome in these patients, thus permitting rational choices among therapeutic options such as adjuvant chemotherapy, hormonal therapy, and the development of novel therapeutics.

To identify prognostic biomarkers that identify subgroups of breast cancer patients with alternative outcomes, we performed a retrospective correlative analysis of several proteins implicated in oncogenesis, using immunohistochemical methods on archival paraffin blocks derived from 116 women with early-stage (stage I; n = 73; 63%) (stage-II; n = 43; 37%) breast cancer who were treated with lumpectomy followed by local radiation therapy and had a median follow-up of 12.4 years (Table 1). Immunostaining data were correlated with distant metastasis-free

EXHIBIT A

survival (DMFS) and overall survival (OS). Among the proteins analyzed were several biomarkers previously suggested to provide prognostic information for breast cancer patients (Estrogen Receptor [ER]; Progesterone Receptor [PR], HER2/Neu, p53, Bcl-2, Bax) and the recently cloned gene BAG-1, a 70 kDa protein that binds Heat Shock Protein (Hsp70) and belongs to a family of molecular chaperones which regulates diverse cellular processes relevant to cancer, including cell division, cell survival, and cell migration (3).

Immunostaining of the invasive component of cancers was scored according to intensity (0-4) and percentage of immunopositive cells (0-100%), evaluating the entire tissue-section, with the pathologist blinded to clinical details. Histo-staining (H)-scores (0-400) were obtained by determining the product of intensity (0-4 scale) and percentage (0-100%). H-score data were displayed as histograms and dichotomized into positive (high) versus negative (low) groups using optimized cut-offs for BAG-1 (H-score$\geq$150), Bcl-2 (H-score$\geq$180), and Bax (H-score$\geq$140). Immunoscoring for ER, PR, p53, and HER2/Neu was performed by established criteria (4).

Compared to normal breast epithelium (NBE) which was often present along with tumor in the same tissue-sections, cytosolic

immunostaining for BAG-1 was clearly upregulated within the invasive breast cancer cells, with elevated levels of protein observed for 77/116 (66%) breast cancer specimens compared with 9/83 (11%) of NBE ($p<0.001$). Thus, roughly two-thirds of early-stage breast cancers contain elevated levels of BAG-1 protein in their cytosol, suggesting that upregulation of BAG-1 represents a tumor-specific event for a subset of these malignancies. Some of the same tumor specimens also contained histologically evident ductal carcinomas in situ (DCIS); high levels of BAG-1 nuclear immunostaining were found in 9/12 (75%) and 6/12 (50%) sections had high levels of cytoplasmic BAG-1 protein levels in DCIS specimens, suggesting that upregulation of BAG-1 can occur as a relatively early event in tumorigenesis and the translocation of protein from the nucleus to the cytoplasm may be important in cellular transformation (Figure 1).

Kaplan-Meier analysis revealed that elevated levels of BAG-1 were significantly associated with longer DMFS ($p<0.001$) and OS ($p<0.001$) (Figure 2). High BAG-1 protein levels were associated with better 10-year DMFS (90% vs. 40%) and OS (84% vs. 40%). Among the other biomarkers evaluated, only Bcl-2 was significant in univariate analysis as a predictor of longer DMFS ($p<0.001$) and OS ($p<0.001$). ER, PR, HER2/Neu, p53,

and Bax were all insignificant in predicting survival for this cohort of patients. Among clinical and pathological variables (age; tumor size; stage; tumor histology), only stage (I Vs II) was significant in univariate analysis predicting survival.

In multivariate models using Cox-regression analysis with variables including BAG-1, Bcl-2, Bax, p53, ER, PR, HER2/Neu, age and clinical stage, only BAG-1 retained statistical significance as a predictor of DFMS ($p = 0.008$) and OS ($p = 0.02$). All other biomarkers failed to reach clear statistical significance and clinical stage was significant only for DFMS ($p = 0.029$) but not OS. Thus, BAG-1 may represent a novel and independent prognostic factor which is associated with favorable outcome in patients with early-stage breast cancer.

Approximately 30% of patients with apparently localized breast cancer probably have micrometastatic disease which is clinically undetectable at the time of diagnosis and which accounts for most instances of distant relapses and disease related deaths. Predictive markers are greatly needed which can help guide the clinician to make treatment related decisions about the necessity for (or lack thereof) adjuvant chemotherapy, hormonal therapy, and new treatments as they become available. The results reported herein indicate that a large number

of early stage primary breast cancers, representing ~two-thirds of cases in this cohort, arise through a pathway that includes upregulation of BAG-1 protein expression. Based on this small pilot study (n = 116), patients whose tumors contain elevated levels of cytosolic BAG-1 protein are more likely to enjoy long-term survival and freedom from distant metastases, compared to those with BAG-1 negative tumors. Additional studies, including prospective analysis, which seek to establish optimal methods for quantifying BAG-1 expression and larger cohorts of patients are needed to firmly establish the overall prognostic utility of BAG-1 testing for women with early-stage breast cancer.

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FIGURE LEGENDS

Figure 1. BAG-1 Immunostaining. Representative photomicrographs are presented showing examples of BAG-1 immunostaining using a BAG-1 monoclonal antibody (5) at low- (top row) and high- (bottom row) power magnification in (A,B) normal mammary gland (note faint staining of cytosol); (C,D) DCIS (note marked upregulation of BAG-1 immunoreactivity in cytosol of in situ cancer cells; arrow); (E, F) a BAG-1 immunopositive invasive cancer; and (G,H) a BAG-1 immunonegative cancer. BAG-1 is known to be present in the nuclei of some types of cells and nuclear immunostaining is normal for breast epithelium, whereas strong cytosolic immunostaining is abnormal here (5). Colorimetric antibody detection involved use of diaminobenzidine (brown) followed by hematoxylin (blue) counterstaining of nuclei.

Figure 2. High BAG-1 Protein Levels Associated with Longer Survival. Kaplan-Meier survival curves containing the proportion of early stage breast cancer patients whose tumors contain high (O) versus low (♀) levels

of BAG-1 protein is plotted against time (years) for distant metastasis - free survival (DMFS) (top) and overall survival (OS) (bottom).

ANCILLARY INFORMATION FOR REFEREES.

Patient Population: We identified 116 early-stage breast cancer patients treated at Yale University School of Medicine between 1973-1993 for whom the primary paraffin tumor blocks were available for analysis (Table 1). All patients were treated by lumpectomy, with or without axillary dissection, followed by radiation therapy to the intact breast using a median dose of 48 Gy followed by an electron boost to the lumpectomy site to yield a total median dose of 64 Gy. There were 17/116 (15%) patients treated with adjuvant systemic chemotherapy and 20/116 (17%) patients treated with tamoxifen therapy. The patients had a median follow-up of 12.4 years, with a minimum follow-up of 4 years. The study was approved by the Human Investigations Committee at the Yale University School of Medicine.

Immunohistochemical Analysis of BAG-1 and Other Proteins:

Immunoscoring was performed for the invasive component of breast cancers. The pathologist (D.C.) scoring the stained specimens was blinded to the clinical histories of the patients. The intensity of immunostaining on each slide was rated on a 4-point scale: 0, none; 1+, light; 2+, moderate; 3+, heavy; and 4+, intense. The percentage of immunopositive tumor cells was determined by counting a minimum of 200 cells from at least 3

representative high-power fields. H-scores were then calculated as the product of intensity (0-4) X distribution (0-100%) with H-scores ranging from 0-400. To set cut-offs for dichotomization of data into high (positive) and low (negative) expression groups, the H-score data for the entire data-set were displayed as dot-histograms with H-score on the x-axis and the number of patient samples having a given H-score on the y-axis. An H-score $\geq$150 was determined by this approach to be appropriate for use as a cut-off for BAG-1 positivity. Individual H-scores were also determined for Bcl-2 (H-score>180), $Bclx_L$ (H-score$\geq$200), BAX (H-score$\geq$140), HER2/Neu (H-score$\geq$25), p53 (H-score$\geq$50), ER (H-score$\geq$75), and PR (H-score$\geq$75) as previously described (4).

Statistical Analysis:

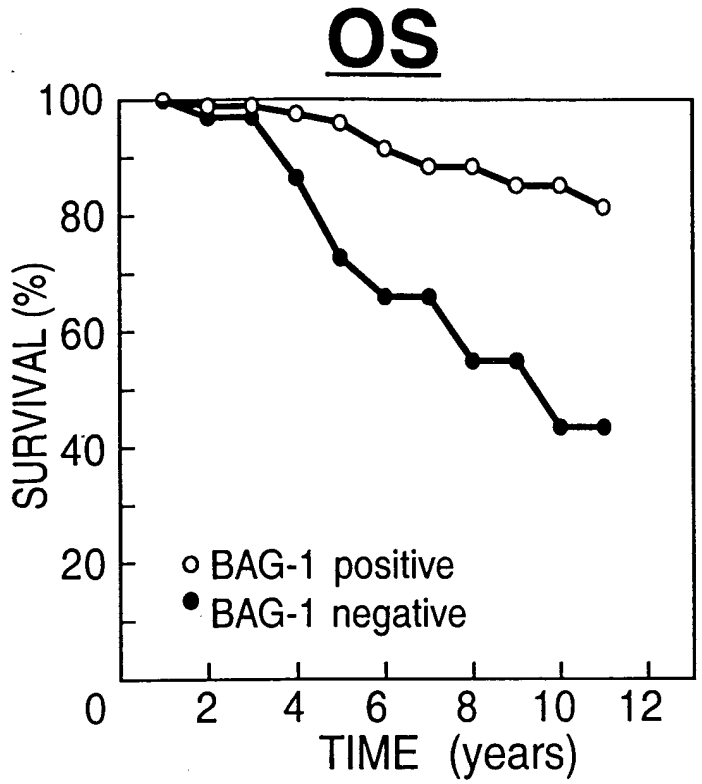
All patient data, including clinical, pathological, and outcome measures were entered into a computerized database using the PRODAS database management system (Conceptual Software Inc., Houston, TX). A multivariate Cox's proportional-hazards regression model was applied to assess whether elevated levels of biomarkers, clinical variables, or pathologic parameters had different effects on DMFS and OS. OS and DMFS were estimated by the Kaplan-Meier method and the log-rank test was used to compare levels of biomarkers, pathologic parameters and

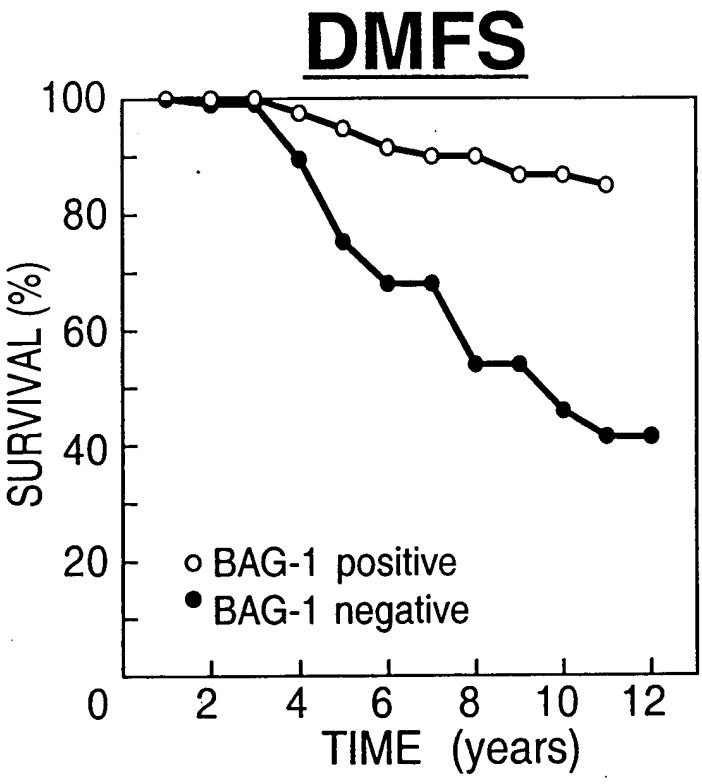
clinical variables in these patients. A p-value of less or equal to 0.05 was considered statistically significant.

Table 1. Characteristics of breast cancer cases

DATA	No.	%
Number of patients	116	NA
Mean age (yrs.)	55	NA
Infiltrating ductal cancer	103	(89%)
Infiltrating lobular cancer	9	(8%)
Infiltrating medullary cancer	4	(3%)
Median follow-up (yrs.)	12.4	NA
Stage I/II Stage I Stage II	116 73 43	(100%) (63%) (37%)
Mean pathologic size (cm)	1.8	NA
Axillary dissection	62	(53%)
Positive lymph nodes	13	(11%)
Estrogen receptor positive	49	(42%)
Adjuvant chemotherapy	17	(15%)
Adjuvant tamoxifen	20	(17%)
Metastatic Disease	35	(30%)

The characteristics of patients used for this study are summarized: number (left column); percentage (right column). Metastatic disease indicates the number (and percentage) of patients who developed clinically detectable metastatic disease after diagnosis and treatment.


OS
100
80
60
40
20
0
SURVIVAL (%)
BAG-1 positive
BAG-1 negative
2
4
6
8
10
12
TIME (years)


DMFS
100
80
60
40
20
0
SURVIVAL (%)
BAG-1 positive
BAG-1 negative
2
4
6
8
10
12
TIME (years)

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ATTACHMENT D

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